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NORTH AMERICAN FROG LUNG FLUKES

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Trematodes from the lungs of anura were first reported over a century ago by Zeder (1800:160). In 1819 Rudolphi (1819:99, 378) described a lung fluke from *Rana esculenta* under the name *Distomum variegatum*. Altho after Rudolphi a number of workers added to the knowledge of this form, it was not until the work of Looss (1894:71) that an adequate description was given. Later the same author (Looss, 1899:600) proposed for the frog lung fluke a separate genus, *Hæmatolæchus*, and upon re-examination of his old *Distomum variegatum* material found that it included three distinct species. The largest of these he designated the type of the genus under the name *H. variegatus* and the others he named *H. similis* and *H. asper*. Stiles and Hassall (1902:20) called attention to the fact that in their opinion Looss (1899:602) in mentioning the name *Distoma simile* in connection with his preparations had given this name to the second of these species before the name *Hæmatolæchus similis*. The name *Distoma simile* which is preempted they call a "still born" homonym and propose for the species the combination *Hæmatolæchus similigenus*. Since the only name proposed by Looss was *Hæmatolæchus similis* this specific name in my opinion should not be superseded. *Hæ-*

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atolæchus asper Looss labels as a species inquirenda since he had only two specimens, but later work has placed the standing of this species beyond any doubt. In 1902 on account of Stål's hemipteron genus *Hæmatolæcha* established in 1874, Looss (1902:732) changed the generic name *Hæmatolæchus* to *Pneumonæces*. He did this influenced by Braun's (1901:55) contention that if family or subfamily names were formed from generic names which differed only in ending, they would be identical. This seems to me to be a logical application of the rule of priority and I shall accept the later name *Pneumonæces*.

Leidy (1851:207; 1856:44) was the first to notice lung flukes in American frogs, but his descriptions are too brief to make precise diagnosis possible. In 1902 Stafford (1902:895) described five new species of this genus from Canada, and in a later publication (1905:687) he designated them *Pneumonæces longiplexus*, *P. breviplexus*, *P. varioplexus*, *P. similiplexus* and *P. medioplexus*. In regard to *P. varioplexus* Stafford (1902:906) writes:

"I have obtained it in Toronto and Montreal, but I find that I have only few mounted specimens and imperfect notes; consequently I shall not describe it at length but shall trust to the drawing to illustrate its chief characters. I shall be glad to come upon it in numbers for greater assurance as to its claims for specific distinction."

It has not been possible to obtain from Stafford this or any other of his type specimens for examination. Except in the size of the eggs the characters of the above species agree with those given for *P. similiplexus*. I have never collected any lung flukes which might be assigned to this species and at present would feel inclined to consider its distinctness as very doubtful. I shall therefore treat it as a species inquirenda. Pratt's (1903:29) *Ostium formosum* Stafford (1905:687) considered to be a synonym of *Pneumonæces medioplexus*. A study of Pratt's type material has confirmed this view. The only other account of frog lung flukes from North America is Seeley's (1906:248) description of *P. complexus*, from *Rana pipiens* in North Carolina. The present paper adds a new species found in Colorado, for which the name *Pneumonæces coloradensis* is proposed. Two other species of

the genus have been described, one by Klein (1905:60) from India as *P. capyristes* and the other by S. J. Johnston (1912:320) as *P. australis* from Australia.

Work on the trematodes of North American frogs was undertaken by the writer in the fall of 1909 while a graduate student at the University of Illinois. Two previous publications (Cort 1912, 1913) have covered part of this work. In addition to the studies on the lung flukes at that time collection and observations have been made of these forms from a number of different localities. Material has been studied of *Pneumonæces longiplexus*, *P. breviplexus*, *P. similiplexus*, *P. medioplexus*, *P. complexus* and of the new species from Colorado, *P. coloradensis*.

I wish to express my appreciation to Professor Henry B. Ward for his guidance during the early part of this work, for the use of his library and for material from his private collections. Thanks are due Dr. George R. La Rue for help in tecnic, and to Dr. H. S. Pratt, Mr. Hermann Douthitt and Dr. M. C. Hall for material.

TECNIC

The worms for sectioning were usually killed after shaking according to Looss' method (Looss, 1901) in saturated corrosive sublimate plus 1 to 3% acetic acid. The specimens which were destined for toto mounts were usually transferred into distilled water before killing, in order that part of the eggs might be evacuated. After this treatment they were killed without shaking. All specimens were left in cold killing fluid from 6 to 18 hours, but when hot fluid was used less time was required. For toto staining Mayer's Paracarmine, Mayer's Hæmalum and Delafield's Hæmatoxylin were found most useful. The worms were much overstained in dilute solutions and then very rapidly and completely destained in 2 to 4% HCl in 70 to 85% alcohol. This method of differentiation has been found useful for most toto mounts of trematodes, since in this way almost all of the parenchymatous tissue is cleared of stain, so that the internal organs stand out clearly. For staining sections hæmatoxylin with eosin as a counter was my favorite combination. Much time can be saved by

staining the worms in toto in Ehrlich's Acid Hæmatoxylin, differentiating on the slide after sectioning and then counterstaining in the higher grades of alcohol. By this method it is not necessary to run the sections on the slide thru a lower grade of alcohol than 85%. The only difficulty in the tecnic of the frog lung flukes is due to the presence of great masses of eggs. In some of the largest specimens sectioning became almost impossible on account of these great masses of eggs. When possible all species were studied alive.

GENERAL DISCUSSION

OCCURRENCE. Altho reports of the occurrence of North American frog lung flukes cover an area including Canada and the north eastern and central portions of the United States, they are relatively so few and scattered that little idea *can* be gained of the range of the genus and the distribution of the several species.

HOST. All reports of North American representatives of the genus *Pneumonæces* are from *Bufo* or *Rana*. There seem however to have been few if any examinations of the smaller anura. A given host may harbor even in a restricted locality several species, and one species may infest several different hosts. *Pneumonæces longiplexus* and *P. breviplexus* have been found in *Rana catesbiana*; *Pneumonæces longiplexus*, *P. similiplexus*, *P. medioplexus*, *P. complexus* and *P. coloradensis* in *Rana pipiens*; and *Pneumonæces medioplexus* and *P. breviplexus* in *Bufo americanus*. In several specimens of *Rana pipiens* which were examined from near Chicago there were found represented three species, *Pneumonæces medioplexus*, *P. similiplexus*, and *P. longiplexus*, in several cases individuals of two different species occurring in the same frog and even in the same lung. Stafford found *P. longiplexus* to be the most common species in *Rana catesbiana*. In my collections from *Rana pipiens*, *Pneumonæces medioplexus* is by far the commonest form. All degrees of infection have been found, varying with the locality from a few flukes in a small proportion of the frogs examined to a heavy infection in a large proportion. The heaviest infection of frog lung flukes ever reported was in a specimen of *Rana pipiens* from Oshkosh, Wisconsin. This frog contain-

ed twenty full grown specimens of *Pneumonæces medioplexus* in one lung and twenty-two in the other. The parasites almost filled the lung cavity, their actual bulk being greater than that of the lung tissue.

SIZE. Within the limits of a given species there is found great variation in the size of sexually ripe individuals, i. e. individuals in which the uterus up to the genital pore contains fully developed eggs. In those species in which a considerable number of individuals were available for study, the largest sexually ripe forms were two and even three times the size of the smallest. Between the extremes were all gradations. Some difference in size seems to be due to differences in environment. The average size of the specimens of *P. longioplexus* which I have collected from *Rana pipiens* is considerably less than that of those collected by Stafford from *Rana catesbiana*. From a region in northern Minnesota all the specimens of *Pneumonæces similiplexus* found, altho crowded with eggs were considerably below the average size for the species. In cases however where different sizes were found in the same locality or host, it seems probable, as Stafford (1902:-896) suggests, that these flukes continue to grow in size after they have reached sexual maturity. Such variations in size within a species make any statements of average size either for the whole animal or for its organs of little value. Suckers and reproductive organs usually vary in size in proportion to the size of the individual, only the size of the eggs and the spines remaining constant. For this reason measurements will always be given for individuals, and mere size comparisons, except in the case of the eggs and spines, will be given little weight in specific diagnosis. However ratios in size such as between oral suckers and acetabulum, and ovary and testes, are found to be quite constant within the species and give some of the most important criteria for specific determination.

SPINES. Great variation in regard to the spines is found among the species of this genus (Fig. 4). Some species are entirely covered with them, some are only partially covered, and in some of the cuticula is entirely smooth. It would seem from the general small size of the spines and their complete absence in

certain species that in the genus *Pneumonæces* they are of little value to the individual and are in the process of degeneration. That there may be variations in this character within a species is suggested by the difference in Stafford's and my own account in regard to the presence and absence of spines in *P. longipectus* and *P. breviplexus* (see specific accounts). In the individuals which I have examined there have been no variations within a species in the character of the spines.

SUCKERS. The acetabulum is always smaller than the oral sucker; in several species it shows degeneration and is apparently of no functional value to the adult.

DIGESTIVE SYSTEM. The characters of the digestive system appear to be of generic value and show specific difference of value for diagnosis only in the ratio in size of the oral sucker to the pharynx and in the length of the esophagus, altho this character is almost entirely obscured by differences in the contraction state of the anterior ends of different individuals.

MALE REPRODUCTIVE SYSTEM. Variations in the size, shape and position of the testes give one of the easiest ways of identifying the species of the frog lung flukes (Figs. 2, 3, 8, 9 and 10). The testes are located diagonally in the body usually back of the middle, with the posterior testis on the same side as the ovary. From the anterior tips of the testes pass forward the vasa efferentia which extend near the dorsal surface of the body forward on each side of the seminal receptacle and unite just in front of the ovary to form the seminal vesicle. The seminal vesicle is a long narrow sac filled with sperms and enclosed for its entire length in a thin walled cirrus sac. Just back of the genital pore the seminal vesicle narrows into an ejaculatory duct, surrounded by a group of unicellular prostate glands, and a short cirrus with thick walls. The cirrus varies with the species from sausage to pear shaped. Both the ejaculatory duct and the cirrus are enclosed in the cirrus sac. When the cirrus is not protruded the male passage opens with the metraterm at the common genital pore. Figures 11 and 12 show the relations of these parts for two of the species. In one specimen of *P. medioplexus* which had been placed in distilled water, the cirrus was extruded and a quantity of sperms ejected. The

length of the extended cirrus was equal to about one-half the width of the body at this region. In one toto preparation of *P. longipectus* the cavity of the cirrus contained ripe eggs. This shows a continuity of passage between the metraterm and the cirrus, and suggests the possibility of self-fertilization. This case is parallel to the one Looss (1894:61, Taf. VI, Fig. 127) notes for *Distomum cygnoides*.

FEMALE REPRODUCTIVE ORGANS. The general position of the ovary and the ducts near it are characters of generic rank. Specific differences are to be found in the size and shape of the ovary and slight variations in the size and position of the ducts. The ovary which is rounded, oval, or irregularly lobed, according to the species, lies to one side of the middle of the body just back of the acetabulum. In all the species the condition of sexual amphitropy is found, the ovary being sometimes on one side of the body and sometimes on the other, and the other genital organs in like relation. Lying in the midline of the body and dorsad of the ovary, covering a considerable area, is a group of unicellular glands, the so-called "shell gland." This gland surrounds the female ducts of this region. After reading Goldschmidt's (1909) paper on the yolk and shell glands of trematodes, in which he ascribes the shell producing function not to the so-called "shell gland" but to the vitelline glands, I was struck by the absolute inadequacy of such a gland as the "shell gland" of the frog lung flukes to furnish the material for the shells of the great numbers of thick shelled brown eggs which distend the uteri in these forms. I agree with Goldschmidt that this gland cannot be the shell gland and will follow his usage in calling it Mehlis' gland. In the ducts of the vitellaria could be distinguished the same shell droplets described by Goldschmidt. These facts force me to the conclusion that in the frog lung flukes as well as in the forms described by Goldschmidt the vitelline glands produce the shell material. The seminal receptacle is round or oval in shape near the mid line of the body ventrad of the ovary and Mehlis' gland and overlapping the posterior portions of both. It is a thin walled sac as large or larger than the ovary and is full of disintegrating sperm cells in the larger specimens. No Laurer's canal is present. The oviduct starts as

a narrow tube from a point on the median dorsal side of the ovary. It passes immediately into Mehlis' gland and receives the duct from the seminal receptacle, which is very short and passes up from the dorsal side of that organ. Just after having been joined by this duct the oviduct is met by the medium duct of the vitellaria from the vitelline reservoir. It then passes thru Mehlis' gland as the oötype which is recognizable by its heavy walls and becomes the beginning of the uterus. The uterus runs forward a ways along the inside of the ovary before it turns backward in its course. The vitellaria are much divided, extending for a considerable distance along each side of the body near the dorsal body wall. At either or both ends there are median groups of acini. The groups are composed of numbers of small acini and are connected by longitudinal ducts. These are gathered from each side just back of the ovary into two median ducts, which join just back of the ovary into the median vitelline reservoir (Figs. 13 and 14) *VD*, *VR*.) While there is much individual variation in the number and arrangement of the groups of acini and the ducts, each species is constant within certain limits. Also the size of the acini and the number in a group vary with the species.

UTERUS. Differences in the arrangement of the uterine coils give a character by which certain species can be readily distinguished. The general course of the uterus is from the ovary to the posterior end and back to the genital pore, filling especially in the larger specimens all the available space between the organs. There may or may not be longitudinal folds from the posterior end outside of the intestinal ceca (Figs. 2, 3, 8, 9, 10). Three of the American species have no lateral longitudinal folds of the uterus outside of the intestinal ceca. These forms agree in having a narrow post-acetabular region, so that the genital organs force the intestinal ceca close to the sides of the body. Those species in which lateral longitudinal folds are present have a distinctly widened posterior body and considerable space between the reproductive organs and the lateral margins. *P. longiplexus* in which these folds reach furthest forward has a body distinctly widened clear up to the pharynx. Since in their development the folds of the uterus force their way into all available space between the

organs, the course of the folds would depend to a considerable extent on the size of the body and the size and shape of the organs. In the development of the narrow forms there is no space outside of the intestinal ceca for the posterior folds of the uterus to grow into, altho one very large specimen of *P. medioplexus* showed contrary to the usual condition in this species short folds outside the intestinal ceca.

EGGS. The uteri in these forms are crowded throughout their lengths with enormous numbers of small eggs. When fully developed they are dark brown and thick shelled and when evacuated contain an embryo in a very early stage of development. Altho in some species the largest eggs may be as large as the smallest or even the average for another species, the average sizes of large numbers of eggs for each species show very distinct differences (Fig. 1, *A, B, C, D, E, F*). In giving the egg size for a species both the range of variation and the average will be recorded. A considerable deviation in the egg size for the species *P. similiplexus* was found in the eggs of three specimens from a single specimen of *Rana pipiens* from Oshkosh, Wisconsin. This variation which has been already noted in another connection (Cort, 1915) is of interest in showing that there may be variations within a species in a character which has up to this time proved so universally constant.

LIFE-HISTORY. Practically nothing is known of the life-history of the frog lung flukes. No account is to be found in the literature of the intermediate host and the development of the cercariæ. Ssinitzin (1907:34) found stages of *P. variegatus* free in the body cavity of both nymphs and adults of the damselfly, *Calopteryx virgo*. By feeding experiments he was able to infect frogs with these forms, showing that the insect here serves as the transfer or secondary intermediate host. Stafford (1902) gives some data on immature forms in frog lungs but makes no attempt to follow out the development of organs. For the species *P. coloradensis* I obtained from a frog's lung a considerable series of different ages which will be considered in connection with the description of that species. A very immature specimen (Fig. 5) suggests from the character of its excretory and digestive systems that the cercariæ of the lung flukes belong to the Xiphidiocercariæ.

SYSTEMATIC SECTION

Pneumonæces Looss 1902

Syn. *Hæmatolæchus* Looss 1899, preoccupied

DIAGNOSIS. Body flattened, elongate, tapering anteriorly; cuticula delicate, wholly or partially covered with spines, or smooth; acetabulum smaller than oral sucker, sometimes very small and of little functional value; digestive system with large oral sucker, no prepharynx, good sized pharynx, short esophagus and long intestinal ceca extending to the posterior end of the body; excretory vesicle Y shaped, with long median stem extending up to the region of the ovary and short branches; genital pore in mid-ventral line just back of the oral sucker; long cirrus sac extending from the acetabulum to the genital pore and enclosing a long seminal vesicle, a short ductus ejaculatorius surrounded by prostate glands, and a short protrusible cirrus; ovary with or without lobes just back of the acetabulum; testes round, oval to elongate, with or without lobes, on opposite sides of the body, back of the ovary; Laurer's canal absent; large seminal receptacle present; vitellaria divided into groups of small acini arranged along the sides of the body from in front of the acetabulum to back of the posterior testis; uterus much coiled extending to the posterior end of the body, in some species with longitudinal folds outside the intestinal ceca; eggs very numerous, small with dark brown shells; habitat in the lungs of Anura.

Pneumonæces longiplexus Stafford 1905

Syn. *Hæmatolæchus longiplexus* Stafford 1902

DIAGNOSIS. Characters of genus; large thick species; cuticula covered in all parts of the body with a dense coating of small spines; oral sucker twice as large as acetabulum; ovary somewhat lobed; testes long and narrow, the posterior extending almost to the posterior end of the body; cirrus pear-shaped; vitellaria of very small acini, with from 16 to 26 in a group; uterus with longitudinal folds outside the intestinal ceca extending to the pharynx; eggs average 0.0248 mm. in length and 0.015 mm. in width; from lungs of *Rana catesbiana* and *R. pipiens*.

DESCRIPTION. *Pneumonæces longiplexus* was found by Stafford (1902:901) to be very common in *Rana catesbiana* Shaw, in Ontario, Quebec, New Brunswick, and Nova Scotia. I have found this species infrequently in *R. pipiens* from near Chicago, Illinois. In several hundred frogs from this region it occurred to the number of fifteen specimens in ten hosts. Also four specimens of this species were found in a bull-frog, *R. catesbiana*, caught near Urbana, Illinois, and others from the same host from North Judson, Indiana.

The specimens of *Pneumonæces longiplexus* from *Rana pipiens* are on the average smaller than those described by Stafford from the bull-frog. Their position in the frog's lung was different from that of any of the other lung flukes found. Instead of hanging by the oral sucker they were coiled up among the folds of the lining of the lung and pushed against the wall so that they were visible externally. As soon as the infected lung was opened under saline solution, they floated away without the tearing of tissue necessary in the loosening of the other species. The freed worms showed considerable power of movement but no locomotion. The pre-acetabular region especially was capable of extension into a long slender neck which was swayed backward and forward.

Well extended toto mounts of this worm are in the form of an elongate ellipse (Fig. 10) with the anterior end bluntly narrowed. The smallest of the mounted specimens was much distended with eggs and had a length of 2.64 mm. and a width of 1.17 mm. An averaged sized mount was 4.6 mm. long and 2 mm. in width. Stafford gives the size of mounted specimens of this species from the bull-frog as 7 to 8 mm. in length and 2 mm. in width. He also notes one living specimen which measured up to 15 mm. in length. This species had a thickness greater than half its width. In one series cross sections measured in the pre-acetabular region 0.93 mm. in width and 0.59 mm. in thickness, thru the ovary 0.96 mm. in width and 0.54 mm. in thickness, and thru the middle of the testicular region 0.86 mm. in width and 0.43 mm. in thickness.

The cuticula in *Pneumonæces longiplexus* for the specimens which I have examined is 0.008 to 0.01 mm. in thickness and is

covered in all regions of the body with a very dense coating of spines, about 0.001 to 0.002 mm. apart and 0.004 to 0.006 mm. in length. The spines point backward and are set only part way thru the cuticula (Fig. 4, *A*). Stafford's account of the cuticula in this species is entirely different from the description given above. He writes (1902:902) of the cuticula of *P. longiplexus*:

"The cuticle in this species is thick and perfectly smooth, there being no trace of spines either in the fresh worms or the preserved sections." Whether this difference is due to a variation within the species or an error in Stafford's account is impossible at the present writing to determine.

The ratio in size in this species of the oral sucker to the pharynx varies from 2:1 to 5:3, and that of the oral sucker to the acetabulum from 5:2 to 2:1. In a large mount 7 mm. in length the oral sucker was 0.36 mm. in length and 0.42 mm. in width, the pharynx 0.22 mm. in length by 0.18 mm. in width and the acetabulum 0.17 mm. in diameter.

In *P. longiplexus* the ovary is just back of the limits of the first third of the body length, and on account of the extreme length of the testes the reproductive field fills practically all of the posterior two-thirds of the worm (Fig. 10). The testes (Fig. 10, *T*) are very characteristic. They are much elongated, and extend almost to the ends of the intestinal ceca, with the one on the same side as the ovary reaching slightly posteriad and being longer than the other. They are irregular cylinders tapering to both ends and more than twice as long as wide. At their anterior ends they taper for about two-fifths of their lengths to the tip from which the vasa efferentia are given off, while at the posterior end they are bluntly pointed. In a mount 4.4 mm. long the posterior testis extended posteriad 0.4 mm. farther than the anterior, within 0.54 mm. of the posterior end of the animal. The posterior testis of this same specimen was 1.26 mm. long by 0.32 mm. wide and the anterior was 1.08 mm. in length and 0.27 mm. in width. In a cross section 0.9 mm. wide by 0.54 mm. thick thru about the middle region of the testes of another worm, the posterior testis measured 0.23 mm. in width and 0.43 mm. in thickness and the anterior 0.20 mm. in width by 0.36 mm. in thickness.

The male ducts show characteristics of specific value in the cirrus which when not protruded is a short thick pear-shaped organ, and in the shortness of the ejaculatory duct (Fig. 12, C).

The ovary (Fig. 10, O) is a somewhat oblong body irregularly lobed, lying a little to one side of the middle of the worm, with its anterior end dorsad of the acetabulum. In a mount 6.8 mm. long the ovary was situated 2.2 mm. from the anterior end with its long axis slightly diagonal to the long axis of the animal, and measured 0.85 mm. in length and 0.72 mm. in width. It is not as thick as in the other species of lung flukes studied. In a cross section thru its middle measuring 1.3 mm. in width and 0.63 mm. in thickness the ovary measured 0.65 mm. in width by 0.22 mm. in thickness.

Lying a little behind and ventrad of the ovary in almost the exact center of the animal is the seminal receptacle (Fig. 10, SR), a regularly oval organ, slightly elongated with its long axis coinciding with the long axis of the worm. It is slightly larger than the ovary and fills the space between it and the ventral body wall, overlapping it for about three-fourths of its length. In a frontal section thru the middle of a specimen 3.6 mm. in length the seminal receptacle measured 0.51 mm. in length by 0.38 mm. in width, and in a transverse section 0.98 mm. in width by 0.37 mm. in thickness it measured 0.32 mm. in width by 0.27 mm. in thickness. The connections of the female reproductive organs of *P. longiplexus* are shown in figure 14.

The vitellaria (Fig. 10, Y) of this form are characterized by the large number of acini in a group, from 16 to 26, and by the small size of the individual acini, which in a specimen of medium size varied from 0.064 mm. to 0.096 mm. in length and from 0.040 to 0.064 mm. in width. The vitellaria extend from a point about half way between the anterior tip and the acetabulum to within a short distance of the posterior extremity. There was to be found considerable individual variation but this could not be worked out in detail, because in most of the specimens available the vitellaria were hidden by great masses of eggs. Figure 10 gives an idea of their arrangement in one specimen.

The uterus (Fig. 10, *U*) passes forward from the oötype around the anterior end of the ovary and then thru the region back of this organ in a series of transverse folds. From just in front of the testes it passes directly back between these organs to the posterior end of the animal, where it makes two very long and voluminous longitudinal folds outside of the intestinal ceca up to the region of the pharynx. From the posterior end of the animal it then passes forward in a series of median coils, more or less voluminous depending on the quantity of eggs present, which are ventral in position and for the most part transverse in direction. Toward its anterior end it narrows into a metraterm about 0.45 mm. in length (Fig. 12, *M*). The uterus is characterized by its large caliber, the simpleness of its folding by the great length of the lateral longitudinal coils outside the intestinal ceca and by the general longitudinal direction of its coils.

Fully developed eggs in this species (Fig. 1, *E*) vary from 0.022 to 0.027 mm. in length and from 0.014 to 0.017 mm. in width. The average of a large number of counts from several different individuals was 0.0248 mm. in length by 0.015 mm. in width.

The above account supplements Stafford's original description of the species. Differences were found in the size of the animals and the presence of spines in my specimens.

Pneumonæces breviplexus Stafford 1905

Syn. *Hæmatolæchus breviplexus* Stafford 1902

DIAGNOSIS. Characters of the genus; largest American frog lung fluke; thickness almost half width; cuticula thick, entirely smooth; oral sucker twice the size of acetabulum; ovary very deeply lobed; testes elongate, usually lobed; acini of vitellaria 12 to 20 in a group; longitudinal folds of uterus outside intestinal ceca reaching in front of posterior testis; eggs average 0.0225 mm. in length by 0.0144 mm. in width; from lungs of *Rana clamitans*, *R. catesbiana* and *Bufo americanus*.

DESCRIPTION. Stafford (1902:904) described *P. breviplexus* from Canada from the lungs of the bull-frog, *Rana catesbiana*. He found this species not nearly so common in this host as *P. longiplexus* and sometimes in the same host as that species. The first

specimens of this species which came to my notice were from the common toad, *Bufo americanus*, from Oklahoma. Later several were taken from the lungs of specimens of *Rana catesbiana* and *R. clamitans* from North Judson, Indiana.

This is the largest American representative of this genus, being approached only by *Pneumonæces longiplexus*. The largest mount which I have is of one of the specimens from *Bufo americanus* which in a somewhat contracted condition measures 9.4 mm. in length and 2.74 mm. in width. In a normal state of contraction the body is spindle shaped, tapering slightly to the blunt posterior end and with the pre-acetabular region somewhat more tapering (Fig. 9). The smallest specimen of this species examined had the uterus in complicated folds crowded with eggs. It had a length of 5.8 mm. and a width of 1.3 mm. in the region of the anterior testis. This mount was used in making the drawing for this species since the structures were less obscured by the uterine coils than in the other specimens mounted. The thickness was about equal to half the width. In a series of cross sections the width at the region half way between the anterior end and the acetabulum was 1.1 mm. and the thickness 0.7 mm.; thru the ovary the width was 1.7 mm. and the thickness 0.85 mm.; and in the region of the anterior testis the width was 1.8 mm. and the thickness 0.7 mm.

The ratio of the oral sucker to both the pharynx and the acetabulum averages about 2:1. The actual size of these structures is of little value in specific diagnosis since they are subject to so much individual variation. In a specimen 9.4 mm. long by 1.4 mm. wide the oral sucker had a transverse diameter of 0.28 mm. and the pharynx 0.145 mm. while in the smallest specimen studied which had a length of 5.8 mm. the oral sucker had a transverse diameter of 0.4 mm. and the pharynx of 0.19 mm. The ratio was found in all cases to vary but slightly and the size usually varied directly with the size of the individuals.

The cuticula in all the specimens of this species which I have examined is very thick and entirely without spines. In a place where there is an average extension the cuticula is from 0.25 to 0.30 mm. in thickness. Stafford (1902:904) writes of the cuticula and spines of this species:

"The cuticle of this species is thick, but unlike the preceding species (*P. longiplexus*) is beset with numerous backward projecting spines." Either the presence of spines is a variable factor or Stafford confused his observations.

In *P. breviplexus* the genital field, without the vitellaria takes up about half the length of the body and extends to within about one-fifth of the body length of the posterior end. The ovary (Fig. 9, *O*) is to one side of the median line and is very deeply lobed on its lateral margin. This deep lobing of the ovary is in marked contrast with the other species of the genus in which this organ is unlobed or very slightly lobed. The seminal receptacle is an unlobed structure ventrad of the ovary and about the same size as that organ. The testes (Fig. 9, *T*) are elongate structures irregularly lobed. Sometimes the lobing is along the median margin and sometimes along the lateral margins. The testes overlap for a part of their length. In the specimen 9.4 mm. long and 2.74 mm. wide the ovary had a length of 1.57 mm. and a width of 0.92 mm. The testes overlapped for the distance of 1 mm. and the posterior extended within 2 mm. of the posterior end. The anterior had a length of 1.71 mm. and a width of 1.03 mm. and the posterior had a length of 2.31 mm. and a width of 1.1 mm.

The vitellaria in this species (Fig. 9 *V*) are very like those of *P. longiplexus*. In a specimen of average size the acini measure 0.08 mm. to 0.12 mm. in length and 0.056 mm. to 0.096 mm. in width. Since the size of the vitellaria varies with the size of the animals these measurements are only of value in broad general comparisons. The number of acini in a group varies from 12 to 20 with an average number of 15 of 16. In a few specimens in which all the vitellaria could be made out the number of groups varied from 16 to 20. The number of individuals studied were too few to make any statement of individual variations or average conditions for these structures of much value. However the general condition shown in Figure 9 held for the five individuals in which these structures could be made out.

The folds of the uterus in *P. breviplexus* are large, as in the previous species. From the oötype the uterus passes posteriad between the testes to the posterior end. From here long longitudi-

nal folds extend outside the intestinal ceca. There is considerable variation in the length of these folds apparently correlated with the number of eggs present. In the individuals where they were shortest they extended in front of the posterior testis and in one case where the worm was much distended with eggs the fold on the same side as the ovary extended to the middle of that structure and the one on the other side in front of its anterior margin. This approaches rather closely to the condition found in *P. longi-plexus*. Stafford must have made his description of this species from a few individuals since he describes the longitudinal folds of the uterus as short and from this character picks the specific name *brevi-plexus*. From the posterior end the ascending part of the uterus coils forward filling the available space between the ovary and the testes. In front of the ovary it passes forward in short transverse folds up to the genital pore, in very large specimens forming a black mass in this region.

The uterus is for its whole length crowded with very small eggs (Fig. 1, *F*). Measurements of fully developed perfect eggs give a variation in length from 0.0205 mm. to 0.026 mm. and in width from 0.013 mm. to 0.016 mm. with an average length of 0.0225 mm. and a width of 0.0144 mm. This is the smallest egg size recorded for any of the species of *Pneumonæces*.

P. longi-plexus and *P. brevi-plexus* are very closely related. They are the largest and heaviest of the frog lung flukes. Both show lobing of the ovary, altho that of *P. brevi-plexus* is much more pronounced, and both have elongate testes. The vitellaria of both show similar arrangement and have small numerous acini in the groups. Further the lateral longitudinal folds of the uterus outside the intestinal ceca are longer in these two species than in any others of the genus and the eggs are very nearly the same size, being the smallest for the genus. Slight differences between the species show in almost every part. The most useful for distinguishing them are the differences found in body shape, length of the lateral longitudinal folds of the uterus and shape of the ovary and testes.

Pneumonæces similiplexus Stafford 1905

Syn. *Hematolæchus similiplexus* Stafford 1902

DIAGNOSIS. Characters of genus; cuticula with scattered spines which thin out back of acetabulum and are entirely absent back of the posterior testis; ratio of oral sucker to acetabulum 4:3; ovary and testes without lobes, small round or oval; acini of vitellaria large with 6 to 13 in a group; longitudinal folds of uterus outside of intestinal ceca reaching beyond posterior testis; eggs average 0.0376 mm. in length by 0.0184 mm. in width; in lungs of *Rana pipiens* and *Bufo americanus*.

DESCRIPTION. The original description of *Pneumonæces similiplexus* is by Stafford (1902:907). He reports this species from Canada from the lungs *Rana virescens* Kalm and from *Bufo lentiginosus* Shaw. I have found it only in *Rana pipiens*, sometimes occurring with *P. medioplexus*, but never as common as that form. This fluke (Fig. 3) is spindle shaped, widest just back of the middle of the body and tapering gradually toward both ends, with the pre-acetabular region somewhat long and narrow. Stafford notes that this species is more inclined to be cylindrical than the other species he studied of this genus. However, all the specimens which I have examined are distinctly flattened, having a thickness less than one-half the width. In the size of sexually mature individuals there is a great variation. In the smallest worms examined the folds of the uterus were crowded with fully developed eggs. One of these specimens measured only 1.8 mm. in length and 0.67 mm. in width at the region of the testes. One of the largest mounts measured 5.8 mm. in length by 1.96 mm. in greatest width. Figure 3 shows an individual of about medium size. Measurements of a series of cross sections show the relation between width and thickness. A cross section thru the middle of the pre-acetabular region had a width of 0.94 mm. and a thickness of 0.48 mm.; at the acetabulum the width was 1.1 mm. and the thickness 0.48 mm.; at the anterior testis the width was 1.22 mm. and the thickness 0.54 mm.; at the posterior testis the width was 1.25 mm. and the thickness 0.54 mm.; and back of the posterior testis the width was 1.1 mm. and the thickness 0.44 mm.

The acetabulum in this species is located about one-third or one-fourth of the distance from the anterior to the posterior end. The ratio of the oral sucker to the acetabulum averages 4:3 and the

oral sucker to the pharynx about 2:1. The ratio in specimens of different sizes varies but little altho the actual size of the suckers varies with the size of the animal.

The cuticula of *P. similiplexus* has a thickness of 0.007 to 0.012 mm. The region in front of the posterior testis is set with small spines which vary from 0.009 to 0.013 mm. in length (Fig. 4, B). These spines are more numerous around the anterior tip and become very scattered back of the acetabulum, and in the region back of the posterior testis the cuticula is entirely smooth. The above description differs considerably from Stafford's account (1902:907) of the cuticula and spines in this species. His description is as follows:

"The cuticle in preserved specimens is about 0.018 mm. thick and is regularly and thickly beset with spines, about 0.022 mm. in length, leaning backward with a slight curvature and extending thru the whole thickness of the cuticula, with the points projecting beyond. Viewed from the surface where one can see great numbers of them together, they appear to be in longitudinal and transverse rows, often 0.015 mm. to 0.02 mm. apart, but sometimes even less, or more, depending on the region and the state of contraction of the animal. As is common they are most abundant at the anterior end."

The genital field (Fig. 3) not including the vitellaria occupies about the third and fourth fifths of the body length. The ovary and testes are round or oval, small compact unlobed structures, which are definitely separated from each other. The small size, regular outline and position of these organs are characteristic of the species. The seminal receptacle is an oval structure ventrad of the ovary in the median line of the worm and overlapping for part if its length the posterior portion of the ovary. In a small sexually mature specimen 1.9 mm. long the ovary measured 0.29 mm. in length by 0.21 mm. in width, the anterior testis 0.34 mm. in length and 0.34 mm. in width and the posterior testis 0.46 mm. in length by 0.34 mm. in width. In a very large specimen 6.4 mm. long the ovary measured 0.46 mm. in length by 0.43 mm. in width, the anterior testis 0.48 mm. in length by 0.45 mm. in width, and the posterior testis 0.56 mm. in length and 0.58 mm. in width. The

posterior testis was in every case larger than the anterior. While the ovary and testes were actually larger in the larger specimens than in the smaller, in proportion to the size of the animal they were always larger in the small individuals. In a section thru the ovary 1.1 mm. wide and 0.54 mm. thick the ovary was 0.26 mm. wide and 0.26 mm. thick. The testes fill almost the entire thickness of the posterior body region. In a cross section thru the posterior testis 1 mm. wide by 0.37 mm. thick the testis had a thickness of 0.33 mm.

The vitellaria (Fig. 3) in *P. similiplexus* are characterized by the large size of the acini and the small number in a group. In average sized specimens the acini have a length varying from 0.096 to 0.14 mm. and a width from 0.06 to 0.1 mm. The number in a group varies from 6 to 13 with the average number about 10. The groups (Fig. 3, Y) extend from about half way between the anterior end and the acetabulum to within a short distance of the posterior tip. There are always at least two groups behind the posterior testis between the intestinal ceca, and two or three median groups at the anterior end. In five specimens where the complete arrangement could be made out there was found to be considerable variation. The total number of groups varied from 16 to 19, with six or seven on a side beside the median groups. Stafford notes that the vitellaria in this species extend to the ends of the intestinal ceca. His drawing however shows them extending only to the region of the posterior testis. Klein (1905:64) at the end of his description of a new species of this genus, sums up the specific differences of all the species of the genus *Pneumonæces* in a table for comparison. As one characteristic of *P. similiplexus* in order to bring it into close relation to *P. similis* Looss he gives the following:

"Dotterstöcke reichen nicht bis zum hindern Rand des hindern Hodens;" but he adds this note:

"Stafford gibt in seiner Arbeit 1902 an, dass die Dotterstöcke bei *Pneumonæces similiplexus* bis ins Hinderende des Körpers, jedenfalls bis zum Ende der Darmschenkel reichen; diese Bemerkung stimmt jedoch nicht mit seiner eignen Abbildung überein, da letzere gleiches Verhalten zeigt wie bei *Pneumonæces similis*."

That Klein should have chosen this characteristic from Stafford's description rather than his drawing is shown by the extent of the vitellaria in the specimens of this species which I have examined.

The folding of the uterus is characteristic. From its beginning in the shell gland the uterus passes for a short distance forward, and then goes to the right side and loops down in a slightly dorsad position to the region back of the posterior testis where it fills the space between the intestinal ceca. From the posterior end of the body it forms two longitudinal folds outside the intestinal ceca, which reach beyond the anterior margin of the posterior testis. The ascending part of the uterus passes forward ventrad of the descending loops and between the testes to the right side of the body where it runs longitudinally for a short distance. Next it passes to the left side just behind the ovary dorsad of the acetabulum and fills with short transverse folds the region between the intestinal ceca up to the genital pore. The above description holds good for those individuals in which the ovary is on the right side of the body. The course of the uterus is exactly reversed when the ovary is to the left.

The eggs of this species are the largest of the American species of the genus (Fig. 1, *A*). Fully developed eggs vary from 0.034 to 0.04 mm. in length and from 0.017 to 0.21 mm. in width with an average of 0.0376 mm. in length and 0.0184 mm. in width.

Pneumonæces medioplexus Stafford 1905

Syn. *Hæmatolæchus medioplexus* Stafford 1902

Ostiolum formosum Pratt 1903

DIAGNOSIS. Characters of genus: long slender worms; cuticula covered with small spines; ratio of oral sucker to acetabulum greater than 4:1; ovary and testes unlobed; acini of vitellaria large with from 6 to 13 in a group; no longitudinal folds of uterus outside intestinal ceca; eggs average 0.032 mm. in length and 0.0186 mm. in width; in lungs of *Rana pipiens*.

DESCRIPTION. *P. medioplexus* was first reported from Canada by Stafford (1902:908) from the lungs of *Rana virescens* Kalm. One year later the same form was described by Pratt (1903:34) as *Ostiolum formosum*. This species has proven to be by far the

most common fluke in the lungs of specimens of *Rana pipiens* which I have examined. I should expect to find it widely distributed throughout the range of *Rana pipiens*. Infection is sometimes very heavy, records showing as many as thirty or forty flukes in one frog. The worms are firmly attached by the oral sucker to the walls of the lung, and their bodies hang free in the cavities. They are very active either when attached or freed, showing great power of extension and contraction of the body, and a very free movement of the pre-acetabular region.

P. medioplexus is an elongate slender worm (Fig. 2) widest at the region of the anterior testis. One of the largest mounts had a length of 7.8 mm. and a width at the region of the anterior testis of 1.2 mm. From the ovary back to the limit of the posterior testis the width is about uniform, the pre-acetabular region is considerably attenuated and the region back of the posterior testis narrows to a bluntly pointed posterior tip. The smallest sexually mature specimen examined had a length of 3.9 mm. and a greatest width of 0.59 mm. Stafford's notes that living specimens of this species may reach a length of 15 or 16 mm.

This worm is distinctly flattened, the thickness being on the average about two-fifths of the width. Measurements of a series of cross sections of a single worm show this relation. In a section in the middle of the pre-acetabular region the width was 0.66 mm. and the thickness 0.33 mm.; at the acetabulum the width was 0.93 mm. and the thickness 0.38 mm.; at the middle of the ovary the width was 0.94 mm. and the thickness 0.42 mm.; thru the middle of the anterior testis the width was 1.1 mm. and the thickness 0.45 mm.; thru the posterior testis the width was 1.12 mm. and the thickness 0.46 mm.; and half way between the posterior testis and the posterior end of the body the width was 0.94 mm. and the thickness 0.38 mm.

The genital field without the vitellaria occupies a little more than the second third of the body length.

The acetabulum is separated by quite a little distance from the anterior margin of the ovary and is in front of the first third of the body length. It is very small being only about one-fourth or one-fifth the size of the oral sucker. In a specimen in which the

oral sucker measured 0.39 mm. by 0.37 mm. the acetabulum had a diameter of 0.08 mm. The pharynx is about three-fourths the size of the oral sucker. In a specimen 7.8 mm. in length the oral sucker had a length of 0.40 mm. and a width of 0.35 mm. and the pharynx had a length of 0.29 mm. and a width of 0.26 mm.

The cuticula of this species measures on the average 0.007 mm. in thickness and is densely covered with a thick coating of spines about 0.01 mm. in length (Fig. 4, C). Stafford very aptly describes them as looking under low power "like a dense coat of short hair." They cover the entire surface of the body and thin out but little toward the posterior end. Pratt (1903:36) makes the absence of spines an important diagnostic character of *Ostiolum formosum* in fact using it as one of the distinguishing characters of his new genus. The examination of his type specimens showed that the cuticula was entirely sloughed off. This would account for the absence of spines in his description.

The testes (Fig. 2, T) are unlobed round or oval with somewhat squared corners and separated sometimes by a distance equal to half the length of one of them. In the large specimen previously mentioned, 7.8 mm. in length the anterior testis is 0.56 mm. long and 0.64 mm. wide and the posterior testis has a length of 0.64 mm. and a width of 0.6 mm. The thickness of the testes is but little less than their width and is practically equal to the thickness of the body. In a cross section thru the middle of the anterior testis 1.1 mm. wide by 0.41 mm. thick the testis had a width of 0.45 mm. and a thickness of 0.36 mm. The relations of the male ducts in this species show little of specific value. Figure 11 shows the ends of both the male and female ducts.

The ovary (Fig. 2, O) is elongate oval, lying a little distance back of the acetabulum either to one side or the other of the body, with its long axis diagonal to the long axis of the worm and its anterior end toward the midline of the body. The shape of the ovary varies considerably and in several cases it was slightly lobed. In the same specimen for which the measurements of the testes were given the ovary had a length of 0.56 mm. and a width of 0.28 mm. This organ has a thickness equal to its width. In a cross section through the middle of the ovary 0.96 mm. wide by 0.40 mm. thick the ovary

had a width of 0.29 mm. and a thickness of 0.30 mm. In about half the specimens examined the ovary was to the right side, in the other half it was to the left. The seminal receptacle is larger than the ovary and lies in the midline of the body posterior and dorsad of the ovary and overlapping it for about half its length. The relations of the ducts of the female organs are shown in Figure 13 and their characters are of generic rather than specific value.

The acini of the vitellaria (Fig. 2, Y) in *P. medioplexus* are large, about the same size as those of *P. similiplexus*, and there are from 6 to 13 in a group. The groups extend from a point about half way between the anterior tip and ovary to the region back of the posterior testis. It was possible to work out in detail the arrangement of the vitellaria in twelve specimens. Altho considerable variation was found in the number of groups and their position, in all specimens the general grouping was the same. Most of the groups are located in lateral lines along the sides of the body. The most anterior of the groups in each line were always about on a level. The line on the same side as the ovary was the shorter, extending only to the posterior limit of the anterior testis. The longer line on the opposite side always extended beyond the posterior testis with usually a median group connected with the last two posterior groups to form a triangle (Fig. 2, Y). One or two median groups were always present in the pre-ovarian field. In the twelve specimens studied the total number of vitellarian groups varied from 18 to 23. The variations in arrangement are to be found in the number in each lateral line and in the number of the median groups. The following table gives the details of arrangement for the twelve specimens.

	1	2	3	4	5	6	7	8	9	10	11	12
Total number	18	20	21	21	22	21	23	21	21	19	18	20
Number on Long Side.....	8	9	8	10	11	10	13	10	10	10	9	11
Number on Short Side.....	8	8	10	8	8	8	8	9	8	7	7	8
Number of Anterior Median Groups	1	2	2	2	2	2	1	2	2	2	2	1
Number of Posterior Median Groups	1	1	1	1	1	1	1	0	1	0	0	0

There is some question in regard to the value of the characteristics of the vitellaria of the trematodes for specific diagnosis.

Barker (1907) finds considerable variation in these structures among certain species of the genus *Opisthorchis*. I have insufficient material for any detailed analysis of the vitellaria for the genus *Pneumonæces*. In the case of *P. medioplexus* where the largest number of specimens could be studied in detail the variations of the vitellaria fall within narrow limits. The general size of the acini, their number in a group and the number of groups are items which, though somewhat variable, yield definite points of specific difference. It may be suggested that the characters of the vitellaria of a trematode must not be given too much weight in the differentiation of species unless the variations have been worked out in a number of individuals.

The arrangement of the folds of the uterus of this species is very characteristic (Fig. 2, *U*). The uterus at its beginning runs ventrad for a short distance and then courses backward in short transverse folds between the testes, and down one side of the post-testicular region and back the other, filling all the space inside of the intestinal ceca. From the posterior limit of the testes the ascending folds pass between these organs ventrad of the descending folds, then forward ventrad of the seminal receptacle and ovary and up to the genital pore, filling the pre-ovarian space in the larger animals with short transverse folds. The general course of the folds is transverse and at the posterior end there are no longitudinal folds outside of the intestinal ceca. In two very large specimens examined there was a variation in this respect, for on each side in these specimens very short longitudinal folds extended outside of the intestinal ceca. These specimens showed the greatest mass of eggs and the most complicated coils of any of the specimens of this species examined. This variation emphasizes the tendency of the uterus in its development to crowd into every available space. The smallest specimen of this species examined was not half the length of the largest individuals but had mature eggs in the uterus up to the genital pore. In this individual the general course of the uterus was like that described above, but the transverse folds were shorter and much less complex.

The measurements of over two hundred eggs from several different individuals give a variation in length from 0.022 to 0.029

mm. and a range in width from 0.013 to 0.017 mm. The average length was found to be 0.0255 mm. and the average width 0.015 mm. (Fig. 1, *D*). Stafford gives the size of the eggs in this species as 0.028 mm. by 0.018 mm. while Pratt gives the measurements of the eggs for his *Ostiolum formosum* as 0.039 mm. by 0.017 mm. This measurement proved to be an error since in the type specimen of *O. formosum* which I examined the size of the eggs was found to fall within the limits for the species as given above. Pratt has since re-measured the eggs for *O. formosum* with the same results. He ascribes his original mistake to a confusion of notes.

The study of Pratt's type specimen of *O. formosum* establishes its identity with the species *Pneumonæces medioplexus*. Since Stafford has priority his specific name stands. There remains to be considered the further question of whether this species should be separated from the genus *Pneumonæces* and be made the basis of a new genus as Pratt has done. At the time of Pratt's description of this form he knew only of the three European species of the genus *Pneumonæces*. The increased knowledge of these forms in my opinion invalidates Pratt's genus *Ostiolum* and forces us to include the form which he would place in this genus in the genus *Pneumonæces*. Pratt separates the new genus *Ostiolum* from *Pneumonæces* on the basis of the following characteristics. "It [*Ostiolum formosum*] differs principally from the genus *Hæmatolæchus* [*Pneumonæces*] in the position of the acetabulum, which is further forward than in that genus, the size of the testes, which are much smaller than in *Hæmatolæchus*, in the arrangement of the uterine folds, which have a general longitudinal direction in *Hæmatolæchus*, and in the length of the excretory vesicle which extends much further forward than in *Hæmatolæchus*. In that genus also the worms are also often covered with spines, while in *Ostiolum* these structures did not appear in any of the specimens examined by me."

An analysis of the above characters shows that if they are to be made the basis of generic distinction, the genus *Pneumonæces* would need to be split into three or four different genera, which would be absurd in view of the fundamental resemblances. The

position of the acetabulum varies slightly in the different species of this genus depending on the amount of attenuation of the anterior end. Variations in the size and the shape of the testes are more properly specific than generic differences. The arrangement of the coils of the uterus seems to depend on the size and shape of the organs and the shape of the body since it grows into all the available space between the reproductive organs. The difference in the length of the median stem and branches of the excretory vesicle is not as great as Pratt's emphasis on that point would suggest and seems to depend on the length and amount of attenuation of the body. In his description of the excretory vesicle of *Ostiolum formosum* Pratt (1903:35) states that the median portion is close to the ventral side of the animal and that the branches are dorsad of the intestinal ceca. The fact is that in all the species of the genus *Pneumonæces* the median stem of the excretory vesicle is near the dorsal body wall and the branches ventrad of the intestinal ceca, and in Pratt's type specimen mentioned several times already there was no variation from the usual condition. The final point of difference made by Pratt in regard to the absence of spines has been shown to be based on an error. But at any rate since in the different species of the genus *Pneumonæces* there is great variation in the character of the spines the distribution of these structures in this genus must be considered to be of specific rather than generic value.

Pneumonæces complexus Seeley 1906

DIAGNOSIS. Characters of the genus; cuticula entirely without spines; ratio of oral sucker to acetabulum about 4:3; ovary and testes irregular in shape and may be slightly lobed; acini of vitellaria large with 12 to 17 in a group and four to six median groups in front of acetabulum; average size of eggs 0.032 by 0.018 mm.; no longitudinal folds of uterus outside of intestinal ceca; habitat lungs of *Rana pipiens*.

DESCRIPTION. Seeley (1906:248) described *Pneumonæces complexus* from the mouth of *Rana pipiens* from North Carolina. Since that time I have obtained several specimens of this species from the lungs of *Rana pipiens* from Raleigh, North Carolina. These specimens were very much distorted and crowded with eggs

so that the internal organs were obscured and so preserved that it proved impossible to section any of them. However enough can be made out to add several points to Seeley's description, correct some errors and to give this form a place as a distinct species of the genus *Pneumonæces*. I will be glad to obtain better material so that the description can be carried further.

The presence of the specimens found by Seeley in the mouth of the host was without doubt an abnormal circumstance due to the fact that the frogs had been killed with chloroform, giving the worms a chance to migrate from the lungs. The normal shape cannot be determined from the distorted specimens at my disposal. The largest of my specimens had a length of 5.2 mm. and a width of 2 mm. Seeley's specimens were not so contracted. He gives measurements of 5.8 mm. in length, 1.7 mm. in width and 0.71 mm. in thickness. In shape he records them as being widest just in front of the middle of the body, tapering slightly to a blunt posterior end and rapidly toward the anterior end. The oral sucker is about twice the size of the pharynx, and bears a ratio to the acetabulum of 4:3. In the specimen 5.2 mm. long the oral sucker had a length of 0.39 mm. and a width of 0.38 mm., the pharynx a length of 0.22 and a width of 0.21 mm., and the acetabulum a diameter of 0.31 mm. The cuticula in this species is entirely smooth. The ovary and testes are rather irregular in shape and slightly lobed. The testes overlap for part of their lengths.

The vitellaria have from 12 to 17 acini in a group and the acini themselves have a length in average sized individuals of 0.08 to 0.14 mm. and a width varying from 0.056 mm. to 0.096 mm. The groups reach from just a little way back of the pharynx to near the posterior end. They show a greater massing of groups in the anterior end than in any of the other species studied, there being 9 or 10 groups in front of the ovary, 4 to 6 of which are median. The total number of groups in two specimens in which they could be completely made out was 22 and 23.

The uterus in this species has no lateral longitudinal folds outside the intestinal ceca.

The eggs of *P. complexus* (Fig. 1, C) have a length varying from 0.030 to 0.035 mm. and a width from 0.017 to 0.020 mm. The average length is 0.032 mm. and the average width is 0.0186 mm.

Strange to say, Seeley makes no special mention of three rather important characteristics of *P. complexus* as described by him. According to his description and figure (Seeley, 1906:248) this worm has no cirrus or cirrus sac, the genital pore to one side of the median line and the yolk glands on the ventral side of the body. Since Seeley seems to ascribe no particular value to the differences noted above, since he very evidently had insufficient and poor material and since his paper gives evidence of superficial observation and lack of grasp of the subject, I should be inclined to ascribe these differences to mistakes in observation. The specimens I have examined of this species are like the others of the genus in these regards.

Pneumonæces coloradensis n. sp.

DIAGNOSIS. Characters of genus; elongate slender worms; thickness greater than half width; spines present only on anterior tip; ratio of oral sucker to acetabulum 5:4; field of genital glands, without vitellaria, less than one-third body length; ovary and testes round to oval to slightly irregular, but unlobed; testicular zones usually abut; groups of vitellaria in front of acetabulum few; no longitudinal folds of uterus outside intestinal ceca; eggs average 0.0344 mm. in length and 0.0195 mm. in width; habitat lungs of *Rana pipiens*.

DESCRIPTION. *Pneumonæces coloradensis* n. sp. was first brought to my attention by a mounted specimen given me in September 1913 by Dr. M. C. Hall from material collected from Boulder, Colorado. Later the same fall I came upon this species again in the lungs of specimens of *Rana pipiens* from a small ditch east of Colorado Springs, Colorado. At this time in all forty-seven specimens were obtained from nine out of seventeen hosts examined. In the summer of 1914 further examination was made of the frogs from the region of Colorado Springs, showing that in *Rana pipiens* this new species was comparatively common. In fact it is the only frog lung fluke found in this region. In one of the frogs examined in the late summer of 1914 fifteen flukes of this species were found,

showing gradations in size from a little beyond the cercarial stage to very large adults. As in other species of the genus there is considerable variation in the size of sexually mature individuals, the largest being as much as twice the length of the smallest. The largest mount examined measured 8.1 mm. in length and 1.55 mm. in width, while the specimen shown in figure 8 with a fully developed uterus and mature eggs up to the genital pore, measured 3.3 mm. in length and 0.54 mm. in width. In shape this species is most like *P. medioplexus*, being narrow for its length but tapering less toward the ends. The region from the anterior groups of the vitellaria almost to the posterior end has a nearly uniform width. The thickness of the body is greater than half the width in every region except back of the posterior testis. A series of sections of a medium sized worm gave the following measurements: a section through the acetabulum was 0.7 mm. wide and 0.49 mm. thick; one through the region half way between the oral sucker and the acetabulum was 0.56 mm. wide and 0.48 mm. thick; through the middle of the anterior testis the width was 0.77 mm. and the thickness 0.48 mm.; through the middle of the posterior testis the width was 0.8 mm. and the thickness 0.5 mm.; and a section through the region half way between the posterior testis and the posterior end had a width of 0.64 mm. and a thickness of 0.3 mm.

The average ratio of the oral sucker to the pharynx is 10:7 and to the acetabulum 5:4. The ratio in both cases is quite constant. The ratio of the oral sucker to the pharynx varies from 8:5 to 5:4 and that of the oral sucker to the acetabulum from 7:6 to 4:3. The size of the suckers and the pharynx in sexually mature worms varies directly with the size of the worms, and there was no relation between the variations in size and of ratio of these structures.

The cuticula in *P. coloradensis* is smooth except at the very anterior tip in the region over the oral sucker and the pharynx. The spines (Fig. 4, *D*) covering this region are thickly set over the oral sucker and thin out rapidly backward, only a few scattered spines showing back of the pharynx. These spines are the largest for any of the species studied, averaging between 0.009 and 0.013

mm. in length and from 0.003 to 0.005 mm. in diameter at their bases. They point strongly backward and have their points curved backward.

The ovary (Fig. 8, *O*) of this species is just behind the acetabulum which is further back than in any of the other forms studied, being just behind the limit of the first third of the body length. The field of the genital organs, without the vitellaria, is characterized in being further back and shorter than in any of the other American representatives of this genus. The distance from the anterior margin of the ovary to the posterior margin of the posterior testis is less than one-third of the total body length.

The ovary is a round or oval unlobed organ which lies just behind the acetabulum to one side or the other of the body. Ventrad of the ovary but somewhat posteriad and overlapping for about one-half its length is the seminal receptacle, which is a round or oval organ lying in the midline of the body, and a little larger than the ovary. The testes (Fig. 8, *T*) are round or oval to almost rectangular structures—close together with the two testicular zones usually abutting. They occupy approximately the fourth fifth of the body length and are only a little to the side of the median line. This position as in *P. medioplexus* depends on the narrowness of the body in this region. The posterior testis is the larger of the two. The size of the reproductive glands varies directly with the size of the individuals and will be given for two different specimens. In the mount 8.1 mm. in length the ovary had a length of 0.49 mm. and a width of 0.42 mm., the anterior testis was 0.64 mm. in length and 0.60 mm. in width and the posterior testis 0.74 mm. in length and 0.86 mm. in width. In a much smaller specimen 4.4 mm. in length the ovary had a length of 0.32 mm. and a width of 0.22 mm., the seminal receptacle had a length of 0.32 mm. and a width of 0.24 mm., the anterior testis had a length of 0.42 mm. and a width of 0.46 mm., and the posterior testis had a length of 0.46 mm. and a width of 0.44 mm. The testes have a thickness but little less than their width and almost equal to the thickness of the body at the testicular region. In a cross section thru the middle of the posterior testis having a width of 0.9 mm. and a thickness of 0.48 mm. the testis was 0.55 mm. in width and 0.46 mm. in thickness.

The arrangement of the ducts of the reproductive organs is similar to that of *P. medioplexus* and offers no points of importance for specific diagnosis.

The vitellaria are almost entirely limited to the lateral zones, there being at most only one median anterior group and one or two posterior median groups which are always considerably to one side of the median line. The acini have about the same average size as those of *P. medioplexus* and there are from 8 to 14 in a group.

The coiling of the uterus is similar to that of the other narrow forms, *P. medioplexus* and *P. complexus*, there being no longitudinal folds outside of the intestinal ceca and the direction of the folds being transverse.

The eggs vary in length from 0.032 to 0.039 mm. and in width from 0.018 to 0.021 mm., with an average length of 0.0344 mm. and an average width of 0.0195 mm.

A considerable series of immature forms of this species was taken from the lungs of the frogs. The youngest specimen found (Fig. 5) which is by far the youngest specimen of the genus ever reported, was found with a number of other specimens of the same species in the lung of a specimen of *Rana pipiens*. There is no question in my mind that this specimen belongs to the species *Pneumonæces coloradensis* since it was found with immature and mature individuals of this species, and no other frog lung fluke has been found near Colorado Springs where this was collected. Since this immature form gives some hint of the character of the cercaria of this species an extended description of its structure will be given.

This specimen (Fig. 5) has a length of 0.4 mm. and a width of 0.15 mm. The acetabulum is back of the center of the body and has a diameter of 0.063 mm. The mouth opening is slightly subterminal. The oral sucker has a length of 0.079 mm. and a width of 0.074 mm. and the pharynx has a length of 0.0496 mm. and a width of 0.039 mm. It is interesting to note that the ratios of the oral sucker to the acetabulum and pharynx fall within the limits of the ratios found for these structures in the adults. The esophagus is very short and in the figure entirely contracted behind the pharynx. The intestinal ceca run almost to the end of the body of the animal and contain blood, showing that the worm had been in

the frog's lung long enough to be feeding on the blood. The excretory pore is at the middle of the posterior tip. It connects with the main part of the excretory bladder by a short narrow tube, 0.016 mm. in length. The bladder consists of the wide median part and two wide lateral divisions extending up to the region of the oral sucker dorsad of the intestinal ceca. In the living animal all parts of this bladder were crowded with highly refractive concretions like those which gather in the excretory systems of cercariæ. This is the only immature stage in which I found those concretions. The excretory bladder resembled very closely the condition found in encysted Xiphidiocercariæ and this character together with the structure of the digestive system suggests that the cercariæ of the species of the genus *Pneumonæces* belong to this group. The locomotion of this immature specimen was watched on a substratum. With the acetabulum firmly attached the anterior end would stretch forward and the oral sucker would take hold. The acetabulum was then loosened and the body contracted. The acetabulum then took hold and the movement was repeated. By a rapid series of these movements this larval form could make considerable progress. This movement is exactly like that of a cercaria on a substratum. Dorsad of the acetabulum are irregular masses of nuclei which are probably the anlage of the reproductive organs. In the drawing (Fig. 5) they are hidden by the acetabulum.

Several larger immature specimens of this species were available for study. The form shown in figure 6 shows considerable increase in size over that shown in figure 5. The intestinal ceca are much distended with frog's blood hiding the excretory vesicle. The anlage of the reproductive system shows definite differentiation into organs. In a larger form (Fig. 7) which appears about ready to produce eggs, but having none in the uterus, the organs are fully developed and differ only in size from those of the sexually mature specimens. The folds of the uterus in this specimen are much less complicated than in those individuals where a quantity of eggs is present.

P. coloradensis is most closely related to *P. complexus*. It has the same shape of the body and general arrangement of the uterus as that species. The size of the eggs and the general relations of

the reproductive organs are more like that species than any other. Our slight knowledge of *P. complexus* makes comparison of the two species in certain characters impossible. Enough is known however to make sure their distinctness. Differences between these two species are noted in the ratios of the oral sucker to the acetabulum and pharynx, and in the size of the eggs. *P. complexus* is entirely without spines, while *P. coloradensis* has spines at the anterior tip. The most noticeable difference is in the arrangement of the vitellaria. In *P. complexus* there are eight to ten groups of vitellaria in front of the acetabulum of which four to six are median groups, while in *P. coloradensis* there are not more than five groups in front of the acetabulum and in none of the specimens examined was there more than one median group.

KEY TO THE AMERICAN SPECIES OF PNEUMONÆCES

- 1(2) Longitudinal folds of the uterus present outside the intestinal ceca reaching at least in front of the posterior testis..... 3
- 2(1) No longitudinal folds of the uterus present outside the intestinal ceca 7
- 3(4) Testes elongate, length at least twice width..... 5
- 4(3) Testes round or oval, length but little greater than width.....
Pneumonæces similiplexus Staf.
- 5(6) Longitudinal folds of the uterus outside the intestinal ceca reaching to the pharynx.
Pneumonæces longiplexus Staf.
- 6(5) Longitudinal folds of the uterus outside the intestinal ceca never reaching in front of ovary.
Pneumonæces breviplexus Staf.
- 7(8) Acetabulum very small, not one-fourth size of oral sucker.
Pneumonæces medioplexus Staf.
- 8(7) Acetabulum three-fourths size of oral sucker..... 9
- 9(10) Not more than two median groups of the vitellaria in front of the acetabulum.
Pneumonæces coloradensis Cort.
- 10(9) Four to six median groups of the vitellaria in front of the acetabulum.
Pneumonæces complexus Seeley

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ABBREVIATIONS USED IN PLATES

A, acetabulum	OT, oötype
C, cirrus	P, pharynx
CS, cirrus sac	PG, prostate glands
DS, duct of seminal receptacle	S, seminal vesicle
E, excretory vesicle	SR, seminal receptacle
G, genital pore	T, testis
I, intestine	U, uterus
M, metraterm	VD, vitelline duct
MG, Mehlis' gland	VR, vitelline reservoir
O, ovary	Y, vitellaria
OS, oral sucker	

EXPLANATION OF PLATES

All drawings were made with a camera lucida. For the sake of clearness the eggs are omitted from the uteri in the toto drawings.

Fig. 1. Eggs of the American species of *Pneumonæces*, average size.

- A. Egg of *Pneumonæces similiplexus*.
- B. " " " *coloradensis*.
- C. " " " *complexus*.
- D. " " " *medioplexus*.
- E. " " " *longiplexus*.
- F. " " " *breviplexus*.

Fig. 2. *Pneumonæces medioplexus*, dorsal view.

Fig. 3. " *similiplexus*, " "

Fig. 4. Cuticular spines of American species of *Pneumonæces*.

- A. Spines of *Pneumonæces longiplexus*.
- B. " " " *similiplexus*.
- C. " " " *medioplexus*.
- D. " " " *coloradensis*.

The cuticula of *Pneumonæces complexus* and *P. breviplexus* is without spines.

Figs. 5, 6, 7. Stages in the development of *Pneumonæces coloradensis*.

Fig. 8. Fully developed *Pneumonæces coloradensis*, ventral view.

Fig. 9. *Pneumonæces breviplexus*, dorsal view.

Fig. 10. " *longiplexus*, " "

Fig. 11. End passages of the reproductive system of *Pneumonæces medioplexus*.

Fig. 12. End passages of the reproductive system of *Pneumonæces longiplexus*.

Fig. 13. Diagrammatic reconstruction of the connections of the female reproductive organs of *Pneumonæces medioplexus*.

Fig. 14. Diagrammatic reconstruction of the connections of the female reproductive organs of *Pneumonæces longiplexus*.

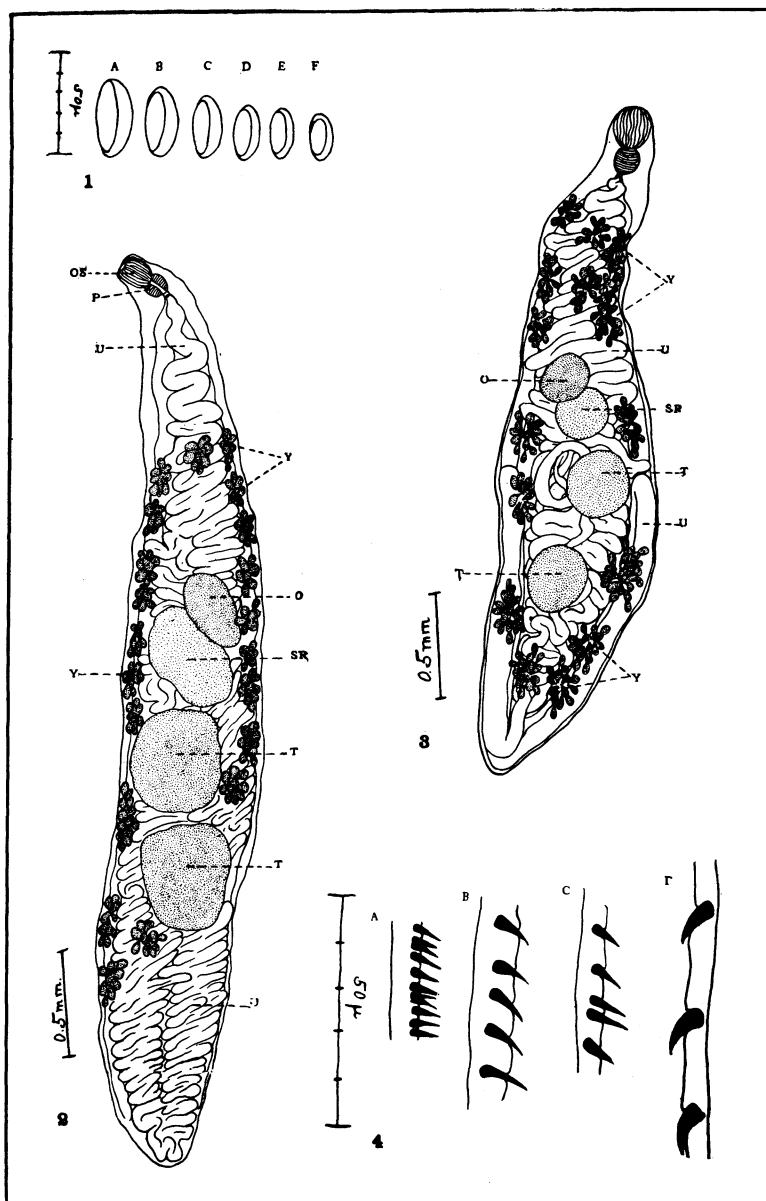


PLATE VII

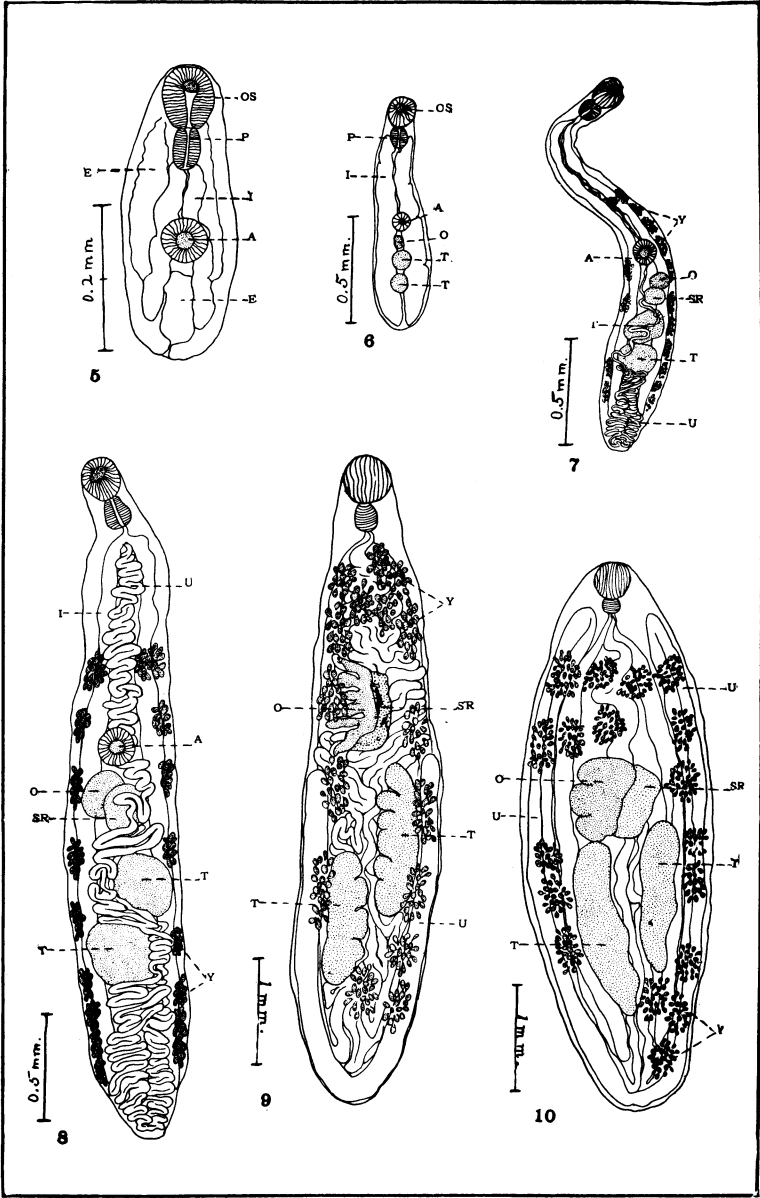
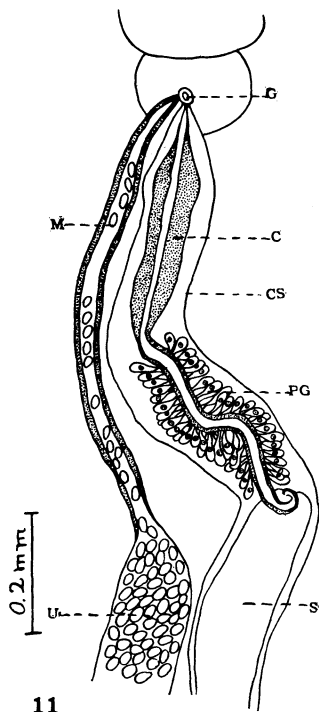
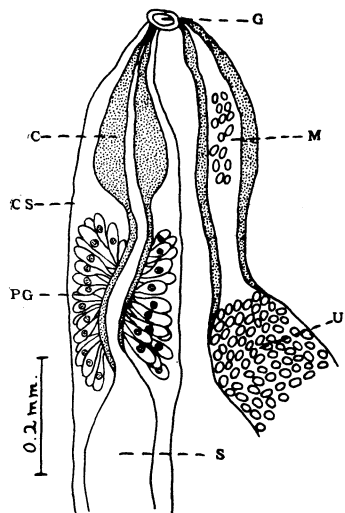


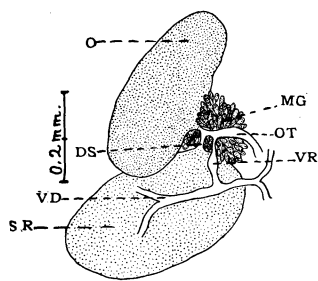
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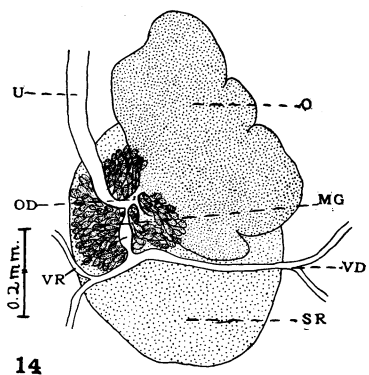
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